IJBST

ISSN: 0974 – 3987
IJBST (2016), 9(1):1-6

Effect of pH, Salinity and Temperature on the Growth of Six Species of Cyanobacteria Isolated from Arabian Sea Coast of Karnataka

Sushanth Vishwanath Rai and Madaiah Rajashekhar
Department of Biosciences, Mangalore University, Mangalagangothri – 574 199, Karnataka, INDIA
bioraitech@gmail.com, profmrajashekhar@yahoo.co.in

ABSTRACT:
Marine cyanobacteria constitute a vast potential resource in various applications such as food, feed, fuel, fertilizer, medicine and in combating pollution. Growth of cyanobacteria cultures are affected by several environmentsl factors. In the present study, the influence of pH, salinity and temperature on the growth of six species of cyanobacteria isolated from Arabian Sea coast of Karnataka was investigated. The axenic cultures were maintained in sterilized f/2 culture media. pH of the media was adjusted to 6.3, 7.5, 8.4, 9.5 and 10.2 with the help of 1 N NaOH and 1 N HCl, whereas salinity was adjusted to 9 ppt, 16 ppt, 25 ppt, 32 ppt and 40 ppt by diluting the media with double-distilled water or by varying the amount of NaCl in the media. The effect of temperature was studied by incubating the cultures at 20˚C, 30˚C, 40˚C and 50˚C. The growth of the isolates were determined in terms of their chlorophyll-a content. All isolated species preferred near neutral to alkaline pH. High pH levels adversely affect the growth of isolates and significantly reduced at pH 10.6. All isolates were able to grow at all salinities. The best growth was seen at 16 ppt and 25 ppt. All isolates showed best growth at 20˚C and 30˚C. The growth has ceased at 40˚C and 50˚C. These factors are useful for establishing the appropriate culture conditions for optimizing the growth of cyanobacteria and also helpful to understand their response to varying environmental factors.
Keywords: Cyanobacteria, pH, Salinity, Temperature, Chlorophyll-a

INTRODUCTION

Cyanobacteria comprise a large component of marine phytoplankton with global distribution [1]. They play a key role in the productivity of oceans and constitute the basis of marine food chain [2]. They tend to form massive blooms under favorable environmental conditions, which include warm water, high nutrient levels and reasonably calm water [3]. Many species of cyanobacteria are known to produce a wide variety of toxins [4].

Cyanobacteria biosynthesize a variety of primary and secondary metabolites such as pigments, phenolics, phycobiliproteins, polysaccharides, amino acids, fatty acids, minerals and vitamins [5-8]. These bioactive metabolites possess diverse biological activities including antibiotic, antioxidant, anti-inflammatory, anticoagulant and anticarcinogenic activities, many with a novel structure have been isolated and characterized [9-14]. Thus, it is essential to identify, isolate and cultivate monocultures of cyanobacteria under controlled laboratory conditions. In the recent years, the emphasis has moved from wild harvests to farming and controlled cultivation of algae to produce valuable new products on a large scale [15]. Formulation of a suitable culture medium and selection of optimum culture conditions are the prerequisite to achieve high biomass production. Chlorophyll-a is the chief photosynthetic pigment of cyanobacteria which can be estimated as a measure of growth [16].

Cyanobacteria exhibit a wide adaptability to environmental factors [17-19]. Ecophysiologica1 studies are helpful in determining environmental conditions which are optimal, favorable or merely tolerable for the growth of phytoplankton [20, 21]. The abiotic factors such as pH, temperature and salinity responses are useful for establishing the appropriate conditions for optimizing cyanobacteria growth [22]. In our previous studies, we have reported the effect of pH, salinity and temperature on the growth of marine phytoplankton namely, *Chroococcus turgidus*, *Lyngbys confervoides*, *Nostoc commune*, *Chaetoceros calcitrans*, *Skeletonema costatum* and *Nannochloropsis oceanica* isolated from Karnataka coastal waters [23]. The effect of environmental factors on the growth and composition of cyanobacteria are studied by Bano and Siddiqui [22], Blackburn et al. [24], Vonshak et al. [25], Sheikh et al. [26], Kumar et al. [27] and Li et al. [28]. The purpose of the present study was to investigate the effect of abiotic factors like pH, salinity and temperature on the growth of six species of cyanobacteria isolated from Arabian Sea coast of Karnataka.

MATERIALS AND METHODS

Isolation and maintenance of cyanobacteria cultures

The six species of cyanobacteria namely, *Oscillatoria fremyii*, *O. geminata*, *O. sancta*, *Phormidium corium*, *P. tenue* and *Spirulina major* isolated from rocks surfaces, puddles and filtered surface sea water. The cyanobactria samples were initially washed with sterilized sea water in the Petri plate to remove the impurities. The samples were diluted and single filament was isolated under the microscope by using micropipette, inoculated into the sterilized f/2 culture media and incubated under 1000 lux illumination at 28±2˚C with 8 : 16 h light and dark regime [29]. After a period of 10 to 14 days of incubation, cultures were microscopically examined for the assessment of growth and contamination. The successful axenic cultures were diluted, subcultured and used for further study.

IJBST

ISSN: 0974 – 3987
IJBST (2016), 9(1):1-6

Experimental design

The salinity and pH of the stock culture media were 30±2 ppt and 7.4±0.5, respectively. The pH of the stock media was adjusted to 6.3, 7.5, 8.4, 9.5 and 10.2 with the help of 1 N NaOH and 1 N HCl. The salinity of the media was adjusted to 9 ppt, 16 ppt, 25 ppt, 32 ppt and 40 ppt by diluting it with double-distilled water or by varying the amount of NaCl in the medium. The temperature range for the growth was determined by incubating the cultures at 20˚C, 30˚C, 40˚C and 50˚C.

The known volume (0.5 ml) of well homogenized culture of each isolate was inoculated into each of the 100 ml conical flasks containing 30 ml of the medium. The cultures were incubated at culture room temperature under illumination of 1000 lux with 8 : 16 h light and dark regime. The cultures were retrieved after the incubation period of 7 days by centrifugation for the determination of growth as increment in chlorophyll-a content. For each culture, the experiments were repeated three times.

Estimation of chlorophyll-a

The cultures were separated from the medium by centrifugation and 10 ml of 90% acetone was added. The tubes were vigoursly shaken and homogenized so as to dissolve completely in the solvent. For complete extraction of the pigments, the tubes were kept in a refrigerator for 24 h. After the extraction period, the samples were centrifuged and the supernatant was collected. The supernatant was made up to 10 ml with 90% acetone and absorbance was measured at 630 nm, 647 nm, 664 nm and 750 nm against 90% acetone as blank. The amount of chlorophyll-a content was calculated using equation given by Jeffrey and Humphrey [30].

$$\text{Chl-a } (\mu g/ml) = (11.85*OD_{664}) - (1.54*OD_{647}) - (0.08*OD_{630})$$

RESULTS

The effect of abiotic factors such as pH, salinity and temperature on the growth of isolated cyanobacteria species was determined by quantifying their chlorophyll-a pigment. The growth of isolates at different pH conditions are shown in Table 1. All isolated species preferred near neutral to alkaline pH. The growth was comparatively lower at pH 6.3. The high pH levels adversely affect the growth of isolates and significantly reduced at pH 10.6 (p<0.05).

The growth of isolated species at different salinities are shown in Table 2. All isolates were able to grow at all salinities. The best growth was seen at 16 ppt and 25 ppt which indicated that isolated species prefer alkaline condition for their optimum growth. But some species namely, *Oscillatoria geminata* and *O. sancta* also exhibited best growth at lower salinity of 9 ppt. At higher salinity of 40 ppt, the growth was drastically decreased.

The effect of the temperature on the growth of isolates is shown in the Table 3. All isolates showed best growth at 20˚C and 30˚C. The growth has ceased at 40˚C and 50˚C. The cyanobacterium *Oscillatoria sancta* was unable to grow at higher temperature. Only *Oscillatoria geminata* and *Phormidium tenue* showed growth at 50˚C.

Table 1: Effect of pH on the growth (chlorophyll-a content in µg/ml) of six species of cyanobacteria.

	pH				
Cyanobacteria	**6.3**	**7.5**	**8.4**	**9.5**	**10.6**
Oscillatoria fremyii	4.09±0.59^{ab}	5.69±0.49^{a}	5.12±0.31^{a}	3.19±0.14^{b}	1.44±0.11^{c}
O. geminata	5.77±0.23^{c}	8.37±0.57^{b}	10.64±0.19^{a}	7.45±0.27^{b}	4.28±0.39^{c}
O. sancta	2.04±0.60^{a}	2.84±0.75^{a}	2.38±0.74^{a}	1.95±0.62^{a}	1.15±0.49^{b}
Phormidium corium	2.40±0.44^{b}	2.37±0.62^{b}	3.54±0.39^{a}	2.08±0.51^{b}	1.23±0.45^{c}
P. tenue	3.43±0.57^{a}	4.08±0.45^{a}	2.97±0.37^{b}	1.82±0.11^{c}	1.16±0.06^{c}
Spirulina major	2.86±0.11^{b}	3.63±0.18^{a}	4.25±0.84^{a}	2.10±0.05^{b}	1.22±0.07^{c}

*Values are means of triplicates ± standard deviations.

** Values across the rows with different superscripts are significantly different (p<0.05).

Table 2: Effect of salinity on the growth (chlorophyll-a content in µg/ml) of six species of cyanobacteria.

	Salinity (ppt)				
Cyanobacteria	**9**	**16**	**25**	**32**	**40**
Oscillatoria fremyii	6.95±0.92^{b}	8.16±0.20^{a}	9.32±0.77^{a}	7.63±0.50^{b}	1.62±0.13^{c}
O. geminata	7.33±0.18^{a}	7.78±0.36^{a}	7.03±0.64^{a}	5.72±0.43^{b}	4.76±0.33^{b}
O. sancta	3.69±0.86^{a}	3.87±0.01^{a}	2.15±0.08^{b}	2.00±0.70^{b}	1.41±0.19^{c}
Phormidium corium	2.42±0.14^{b}	3.11±0.20^{ab}	4.32±0.66^{a}	2.38±0.20^{b}	0.65±0.12^{c}
P. tenue	2.47±0.09^{c}	5.61±0.41^{a}	2.73±0.14^{c}	3.51±0.21^{b}	3.16±0.89^{b}
Spirulina major	3.38±0.45^{b}	4.57±0.64^{a}	2.24±0.29^{c}	1.47±0.08^{c}	0.20±0.06^{d}

*Values are means of triplicates ± standard deviations.

** Values across the rows with different superscripts are significantly different (p<0.05).

Table 3: Effect of temperature on the growth (chlorophyll-a content in µg/ml) of six species of cyanobacteria.

Cyanobacteria	Temperature (˚C)			
	20	30	40	50
Oscillatoria fremyii	7.56±0.25[a]	4.42±0.10[b]	2.69±0.27[c]	-
O. geminata	5.69±0.71[b]	10.69±0.23[a]	4.01±0.20[b]	1.19±0.01[c]
O. sancta	6.75±0.12[a]	2.62±0.03[b]	-	-
Phormidium corium	0.42±0.06[c]	2.91±0.14[a]	1.26±0.01[b]	-
P. tenue	6.36±0.45[a]	3.03±0.14[b]	2.25±0.04[b]	0.89±0.01[c]
Spirulina major	13.79±0.66[a]	6.59±0.03[b]	4.86±0.67[c]	-

*Values are means of triplicates ± standard deviations.
** Values across the rows with different superscripts are significantly different (p<0.05).

DISCUSSIONS

In the present study, the cyanobacteria species namely, *Oscillatoria fremyii*, *O. sancta* and *Phormidium tenue* showed maximum growth at pH 7.5, whereas *Oscillatoria geminata*, *Phormidium corium* and *Spirulina major* showed maximum growth at pH 8.4. The pH value lower or higher than these values was associated with their decreased growth. All isolates also exhibited growth at pH 6.3 but it was very low. The best growth of cyanobacteria isolated from marine habitats in pH range 6.5 to 8.5 was reported by Nagle et al. [18]. Similarly, Konopka [31] observed the highest photosynthetic rates for *Oscillatoria rubescens* in natural populations between pH 6.5 to 8.5. The study conducted by Abdul-Jabbar [21] reported the adverse effect of pH less than 7.5 and higher than 9 on the growth of *Oscillatoria agardhii* and also observed that filaments of cyanobacterium were broken up into smaller filaments when pH was lower than 6. The optimum growth of cyanobacteria at pH <8.5 observed by Muruga et al. [32].

According to Chen and Durbin [33], decrease in both growth rate and photosynthesis of marine phytoplankton at high pH may have been caused by trace metal toxicity or limitation, reduced nutrient availability or changes in the availability of carbon substrate. At high pH, a consistent increase of cell division was differentially regulated in marine phytoplankton [34]. At high or low pH, cells may have to spend energy for maintainance of an internal pH necessary for cell function [35]. Hinga [36] reviewed the effects of pH on marine phytoplankton. He concluded that at extreme pH, only species with a tolerance for high or low pH would grow and dominate the community. Some species have maximum growth near equilibrium pH and others have a range of pH, encompassing equilibrium pH, where growth rate is not affected by changes in pH.

Earlier studies related to pH effect on the growth of cyanobacteria has revealed that pH between 7.4 and 8 is favorable for the optimum growth of cyanobacteria species [22, 37]. The fact that all cyanobacteria were able to grow in acidic (pH 6.5) medium indicates that cyanobacteria can adapt to variable pH conditions as suggested by Buck and Smith [38] and Burja et al. [39]. Wangwibulkit et al. [40] reported that pH higher than 9 or lower than 6 could inhibit the photosynthesis and adversely affect the morphology of cyanobacteria.

Although the isolates preferred alkaline condition for their maximum growth, the growth was significantly decreased at higher salinities. According to Nakanishi and Monshi [41] both photosynthesis and respiration decreases with increasing salinity, but salinity affects photosynthesis more intensively than respiration. Earlier studies have reported that the cultivation of cyanobcteria with higher saline concentrations had lower chlorophyll contents [21, 25]. It has also been reported that chlorophyll is the primary target to salt toxicity limiting net assimilation rate, resulting in reduced photosynthesis and reduced growth [42].

As per earlier reports, cyanobacteria can adapt to the variations in salinity [17, 43] but all cyanobacteria are not halotolerant [44]. Many marine forms can survive at lower salinity, but for their optimum growth they express specific requirements for additional salts [37]. The ability of cyanobacteria to grow with increased Na^+ may be related to their ability to adjust respiration [45], regulate intake or efflux of Na^+ [46], enhance cyclic electron transfer via Photosystem I [47] and produce organic osmolytic compounds [48]. Wide range (0-100%) of salinity tolerance was noticed in *Phormidium tenue*, *Synechococcus cedrorum* and *S. pevalekii* by Nagle et al. [18].

Fu and Bell [49] observed the growth of *Trichodesmium* GBRTRLI101 over a range of salinities from 22 to 43 psu with maximum growth rates and cell yields occurring in the range 33 to 37 psu. No active growth was observed at lower salinity (18 psu). They also noted that large irregular filament aggregates or bundles formed early within the exponential growth phase with low salinities (<33 psu). Effect of salinity on the growth and toxin production of harmful cyanobacterium *Microcystis aeruginosa* was investigated by Liu [50] and found that increased salinity (0 to 20 ppt) decreased the growth and toxin production. Studies of Paerl et al. [51] have indicated that the salinity higher than 0.5 to 2 psu was inhibitory for growth and CO_2 fixation of the toxic cyanobacterium *Microcystis aeruginosa*.

Sellner et al. [52] observed that increasing salinity caused the aggregation of *Microcystis* sp. They suggested that this aggregation phenomenon probably resulted from decreased photosynthetic ability and substitution of divalent cations between hydrophilic side groups of mucilage surrounding the cells. Tel-Or [53] examined the effects of salinity on nitrogenase and related enzyme activities in the cyanobacterium *Calothrix scopulorum* and found that electron transfer via the ferredoxin: ferredoxin-NADP reductase was very salt sensitive.

In the present study all isolates showed active growth at 20˚C and 30˚C, whereas high temperature significantly reduced the growth. Elevated temperature may affect membrane fluidity and denature proteins, which can also lead to a decline in photosynthetic efficiency [54]. Fogg and Thake [55] stated that low growth rate of phytoplankton could be a result of the increase in respiration due to rise in temperature above the species optimum level. The species namely, *Oscillatoria fremyii*, *O. sancta*, *Phormidium tenue* and *Spirulina major* showed good growth at 20˚C, whereas *Oscillatoria geminata* and *Phormidium corium* exhibited high growth at 30˚C. Most of the phytoplankton species (tropical or subtropical) grow best at temperatures ranging from 16 to 27˚C [56]. The optimum is about 24˚C. Most marine cyanobacteria exist in temperatures ranging from -5 to 35˚C, exhibiting temperature optima in the range of 25 to 35˚C [57]. Robarts and Zohary [58] reported that growth rate of cyanobacteria (*Anabaena*, *Aphanizomenon*, *Microcystis* and *Oscillatoria*) were temperature dependent with optima usually at 25˚C or greater. Tsujimura et al. [59] studied the effect of temperature on the growth of bloom forming cyanobacterium *Aphanizomenon flos-aquae* and observed the growth at above 8˚C with an optimum temperature ranging from 23 to 29˚C . Kumar et al. [27] found that *Spirulina platensis* has a wide range of temperature tolerance from 20 to 40˚C. Muruga et al. [32] observed the optimum growth of cyanobacteria at room temperature (23±2℃) and also at high temperature (50℃). Earlier, Goldman [60] reported that the response of phytoplankton to different temperatures is strongly species dependent. The impact of temperature rise can be either beneficial or harmful, depending on nutrient conditions, the physiological stage of the phytoplankton community and the species composition [61].

CONCLUSION

Marine cyanobacteria are considered to be a promising source of high value compounds for the pharmaceutical and food industry. Thus it is essential to identify, isolate and cultivate monocultures under controlled laboratory conditions. The study on the effect of abiotic factors like pH, salinity and temperature on the growth of the isolates are useful for optimizing cell growth and is necessary to understand their response to varying environmental factors.

ACKNOWLEDGEMENTS

The authors are thankful to the Ministry of Earth Sciences, Government of India, New Delhi for the financial assistance and Dr. C. Krishnaiah, Co-ordinator, OASTC, Mangalore University for his help during the study period.

DECLARATIONS

The authors acknowledge that the manuscript submitted is their own original work. All authors participated in the work in a substantive way. All authors have seen and approved the manuscript as submitted. The manuscript has not been published and is not being submitted or considered for publication elsewhere.

REFERENCES

[1] Gallon J.R., D.A. Jones, T.S. Page (1996) *Trichodemium*, the paradoxical diazotroph. Algol Stud, 83: 215-243.

[2] Senthilkumar T., S. Jeyachandran (2006) Effect of salinity stress on the marine cyanobacterium *Oscillatoria acuminata* Gomont with reference to proline accumulation. Seaweed Res Utiln, 28: 99-104.

[3] Udeh P.J. (2004) A Guide to Healthy Drinking Water. iUniverse, Inc., Lincoln, NE.

[4] Paerl H.W., T.G. Otten (2013) Harmful cyanobacterial blooms: causes, consequences and controls. Microb Ecol, 65: 995-1010.

[5] Pugh N., S.A. Ross, H.N. El-Sohly, M.A. El-Sohly, D.S. Pasco (2001) Isolation of three high molecular weight polysaccharide preparations with potent immunostimulatory activity from *Spirulina platensis*, *Aphanizomenon flos-aquae* and *Chlorella pyrenoidosa*. Planta Med, 67: 737-742.

[6] Shalaby E.A. (2011) Algae as promising organisms for environment and health. Plant Signal Behav, 6: 1338-1350.

[7] Priyadarshani I., B. Rath (2012) Commercial and industrial applications of microalgae – a review. J Algal Biomass Utln, 3: 89-100.

[8] Morais M.G., B.S. Vaz, E.G. Morais, J.A.V. Costa (2015) Biologically active metabolites synthesized by microalgae. BioMed Res Int, 2015: 1-15.

[9] Gerwick W.H., L.T. Tan, N. Sitachitta (2001) Nitrogen containing metabolites from marine cyanobacteria. In: Cordell G. (ed.) The Alkaloids. Academic press, San Diego, USA. pp. 75-184.

[10] Luesch H., R. Pangilinan, W.Y. Yoshida, R.E. Moore, V.J. Paul (2001) Pitipeptolides A and B, new cyclodepsipeptides from the marine cyanobacterium *Lyngbya majuscula*. J Nat Prod, 64: 304-307.

[11] Tan L.T. (2006) Biomedical potential of marine cyanobacteria. J Coast Dev, 9: 129-136.

[12] Volk R.B., F.H. Furkert (2006) Antialgal, antibacterial and antifungal activity of two

metabolites produced and excreted by cyanobacteria during growth. Microbiol Res, 161: 180-186.

[13] Abed R.M.M., S. Dobretsov, K. Sudesh (2009) Applications of cyanobacteria in biotechnology. J Appl Microbiol, 106: 1-12.

[14] Pumas C., P. Vacharapiyasophon, Y. Peerapornpisal, P. Leelapornpisid, W. Boonchum, M. Ishii, C. Khanongnuch (2011) Thermostablility of phycobiliproteins and antioxidant activity from four thermotolerant cyanobacteria. Phycol Res, 59: 166-174.

[15] Cardozo K.H., T. Guaratini, M.P. Barros, V.R. Falcao, A.P. Tonon, N.P. Lopes, S. Campos, M.A. Torres, A.O. Souza, P. Colepicolo, E. Pinto (2007) Metabolites from algae with economical impact. Comp Biochem Physiol Part C, 146: 60-78.

[16] Gitelson A.A., S. Laorawat, G.P. Keydan, A. Vonshak (1995) Optical properties of dense algal cultures outdoors and its application to remote estimation of biomass and pigment concentration in *Spirulina platensis*. J Phycol, 31: 828-834.

[17] Thajuddin N., G. Subramanian (2005) Cyanobacterial biodiversity and potential applications in biotechnology. Curr Sci, 89: 47-57.

[18] Nagle V.L., N.M. Mhalsekar, T.G. Jagtap (2010) Isolation, optimization and characterization of selected cyanophycean members. Indian J Mar Sci, 39: 212-218.

[19] Shruthi M.S., M. Rajashekhar (2014) Effect of salinity and pH on the growth and biomass production in the four species of estuarine cyanobacteria. J Algal Biomass Utln, 5: 29-36.

[20] Sigaud T.C.S., E. Aidar (1993) Salinity and temperature effects on the growth and chlorophyll-a content of some planktonic algae. Bol Inst Oceanogr, 41: 95-103.

[21] Abdul-Jabbar A. (2011) The effect of salinity and pH on growth of *Oscillatoria agardhii* Gom. J Coll Educat Pure Sci, 1: 82-89.

[22] Bano A., P.J.A. Siddiqui (2004) Characterization of five marine cyanobacterial species with respect to their pH and salinity requirements. Pak J Bot, 36: 133-143.

[23] Sushanth V.R., M. Rajashekhar (2014) Effect of pH, salinity and temperature on the growth of six species of marine phytoplankton. J Algal Biomass Utln, 5: 55-59.

[24] Blackburn S.I., M.A. McCausland, C.J.S. Bolch, S.J. Newman, G.J. Jones (1996) Effect of salinity on growth and toxin production in cultures of the bloom-forming cyanobacterium *Nodularia spumigena* from Australian waters. Phycologia, 35: 511-522.

[25] Vonshak A.N., K.B. Bunang, M. Tanticharoen (1996) Role of light and photosynthesis on the acclimation process of the cyanobacteria *Spirulina platensis* to salinity stress. J Appl Phycol, 8: 119-124.

[26] Sheikh T.A., Z.A. Baba, P. Sofi (2006) Effect of NaCl on the growth and physiological traits of *Anabaena cylindrica* L. Pak J Biol Sci, 9: 2528-2530.

[27] Kumar M., J. Kulshreshtha, G.P. Singh (2011) Growth and biopigment accumulation of cyanobacterium *Spirulina platensis* at different light intensities and temperature. Braz J Microbiol, 42: 1128-1135.

[28] Li M., P.N. Nkrumah, M. Xiao (2014) Biochemical composition of *Microcystis aeruginosa* related to specific growth rate: insight into the effects of abiotic factors. Inland Waters, 4: 357-362.

[29] Andersen R.A. (2005) Algal Culturing Techniques. Elsevier Academic Press, Burlington.

[30] Jeffrey S.W., G.F. Humphrey (1975) New spectrophotometric equations for determining chlorophylls a, b, c_1 and c_2 in higher plants, algae and natural phytoplankton. Biochem Physiol Pflanzen, 167: 191-194.

[31] Konopka A. (1981) Influence of temperature, oxygen and pH on a metalimnetic population of *Oscillatoria rubescens* Appl Environ Microbiol, 42: 102-108.

[32] Muruga B.N., J.M. Wagacha, J.M. Kabaru, N. Amugune, S.M. Duboise (2014) Effect of physicochemical conditions on growth rates of cyanobacteria species isolated from Lake Magadi, a Soda lake in Kenya. WebPub J Sci Res, 2: 41-50.

[33] Chen C.Y., E.G. Durbin (1994) Effects of pH on the growth and carbon uptake of marine phytoplankton. Mar Ecol Prog Ser, 109: 83-94.

[34] Hansen P.J. (2002) Effect of high pH on the growth and survival of marine phytoplankton: implications for species succession. Aquat Microb Ecol, 28: 279-288.

[35] Raven J.A., W.J. Lucas (1985) Energy costs of carbon acquisition. In: Lucas W.J., J.A. Berry (eds.) Inorganic Carbon Uptake by Aquatic Phytosynthetic Organisms. American Society of Plant Physiologists, Rockville. pp. 305-324.

[36] Hinga K.R. (2002) Effects of pH on coastal marine phytoplankton. Mar Ecol Prog Ser, 238: 281-300.

[37] Rippka R., J. Deruelles, J.B. Waterbury, M. Herdman, R.Y. Stainer (1979) Generic assignments, strain histories and properties of pure cultures of cyanbacteria. J Gen Microbiol, 111: 1-61.

[38] Buck D.P., G.D. Smith (1995) Evidence for a Na^+/H^+ electrogenic antiporter in an alkaliphilic cyanobacterium *Synechocystis*. FEMS Microbiol Lett, 128: 315-320.

[39] Burja A.M., E. Abu-Mansour, B. Banaigs, C. Pyari, J.G. Burgess, P.C. Wright (2002) Culture of marine cyanobacterium, *Lyngbya majuscula* (Oscillatoriaceae), for bioprocess intensified production of cyclic and linear lipopeptides. J Microbiol Methods, 48: 207-219.

[40] Wangwibulkit S., C. Limsuwan, N. Chuchird (2008) Effect of salinity and pH on the growth of Blue-green algae, *Oscillatoria* sp. and *Microcystis* sp., isolated from pacific white shrimp (*Litopenaeus vannamei*) ponds. Kasetsart Univ Fish Res, 32: 1-9.

[41] Nakanishi M., M. Monshi (1965) Effect of variation in salinity on photosynthesis of phytoplankton growing in estuaries. J Fac Sci Tokyo Univ, 9: 19-42.

[42] Rai A.K., G. Abraham (1993) Salinity tolerance and growth analysis of the cyanobacterium *Anabaena doliolum*. Bull Environ Contamin Toxicol, 51: 724-731.

[43] Joset F., R. Jeanjean, M. Hagemann (1996) Dynamics of the response of cyanobacteria to salt stress: deciphering the molecular events. Physiol Plant, 96: 738-744.

[44] Blumwald E., E. Tel-Or (1982) Osmoregulation and cell composition in salt-adaptation of *Nostoc muscorum*. Arch Microbiol, 132: 168-172.

[45] Gabbay-Azaria R., M. Schonfeld, S. Tel-Or, R. Messinger, E. Tel-Or (1992) Respiratory activity in the marine cyanobacterium *Spirulina subsalsa* and its role in salt tolerance. Arch Microbiol, 157:183-190.

[46] Molitor V., W. Erber, G.A. Peschek (1986) Increased levels of cytochrome oxidase and sodium-proton antiporter in the plasma membrane of *Anacystis nidulans* after growth in sodium-enriched media. FEBS Lett, 204: 251-256.

[47] Jeanjean R., S. Bedu, M. Havaux, H.C.P. Matthijs, F. Joset (1998) Salt-induced photosystem I cyclic electron transfer restores growth on low inorganic carbon in a type 1 NAD(P)H dehydrogenase deficient mutant *Synechocystis* PCC6803. FEMS Microbiol Lett, 167: 131-137.

[48] Reed R.H., L.J. Borowitzka, M.A. Mackay, J.A. Chudek, R. Foster, S.R.C. Warr, D.J. Moore, W.D.P. Stewart (1986) Organic solute accumulation in osmotically stressed cyanobacteria. FEMS Microbiol Rev, 39: 51-56.

[49] Fu F.X., P.R.F. Bell (2003) Effect of salinity on growth, pigmentation, N_2 fixation and alkaline phosphatase activity of cultured *Trichodesmium* spp. Mar Ecol Prog Ser, 257: 69-76.

[50] Liu Y. (2006) Effects of salinity on the growth and toxin production of a harmful algal species, *Microcystis aeruginosa*. J U S SJWP, 1: 91-110.

[51] Paerl H.W., J. Tucker, P.T. Bland (1983) Carotenoid enhancement and its role in maintaining bluegreen algal (*Microcystis aeruginosa*) surface blooms. Limnol Oceanogr, 28: 847-857.

[52] Sellner K.G., R.V. Lacouture, C.R. Parrish (1988) Effects of increasing salinity on cyanobacteria bloom in the Potomac river estuary. J Plankton Res, 10: 49-61.

[53] Tel-Or E. (1980) Response of N_2-fixing cyanobacteria to salt. Appl Environ Microbiol, 40: 689-693.

[54] Falk S., D.P. Maxwell, D.E. Laudenbach, N.P.A. Huner (1996) Photosynthetic adjustment to temperature. In: Baker N.R. (ed.) Advances in Photosynthesis and Respiration. Kluwer, Dordrecht, The Netherlands. pp. 367-835.

[55] Fogg G.E., B. Thake (1987) Algal Cultures and Phytoplankton Ecology. The University of Wisconsin Press, Madison,WI, USA.

[56] Creswell L. (2010) Phytoplankton Culture for Aquaculture Feed. Southern Regional Aquaculture Center (SRAC) Pub. No. 5004, Department of Agriculture, US.

[57] Fogg G.E., W.D.P. Stewart, P. Fay, A.E. Walsby (1973) The Blue-Green Algae. Academic Press, London.

[58] Robarts R.D., T. Zohary (1987) Temperature effects on photosynthetic capacity, respiration and growth rates of bloom-forming cyanobacteria. New Zeal J Mar Fresh Res, 21: 391-399.

[59] Tsujimura S., K. Ishikawa, H. Tsukada (2001) Effect of temperature on growth of the cyanobacterium *Aphanizomenon flos-aquae* in lake Biwa and lake Yogo. Phycol Res, 49: 275-280.

[60] Goldman J.C. (1977) Biomass production in mass cultures of marine phytoplankton at varying temperatures. J Exp Mar Biol Ecol, 27: 161-169.

[61] Keskitalo J. (1987) Carbon fixation of phytoplankton species in cooling water of the Olkiluoto nuclear power station, West coast of Finland. Ann Bot Fennici, 24: 23-34.